CURRENT PROGRESS

# Interferon: A Changing Picture

R. P. BRYCE LARKE, M.D.,* *Toronto*

ABSTRACT

**Concepts regarding the nature and function of interferon have undergone considerable modification since its initial description in 1957. A low-molecular-weight protein, interferon has been produced by a variety of host cells following exposure not only to most viruses but also to bacterial cells and endotoxins, rickettsiae, nucleotides and a polyanionic polysaccharide (Statolon). Interferon production and activity require *de novo* synthesis of cellular RNA and protein, although interferon induced *in vivo* by endotoxins appears to involve release from a preformed state.**

**The pathogenesis of primary viral infections may be determined largely by non-immune defence mechanisms. Interferon, detectable in the host's serum and associated with leukocytes during the course of viral illness, may make an important contribution to recovery. Low toxicity, weak antigenicity and wide range of antiviral activity make interferon an attractive therapeutic possibility. Stimulation of inherent interferon-producing mechanisms by administration of relatively innocuous agents may prove beneficial in humans.**

SOMMAIRE

**Depuis que l'interféron a été décrit pour la première fois en 1957, les principes relatifs à sa nature et sa fonction ont subi des changements considérables. Protéine de faible poids moléculaire, l'interféron a été produit par une grande variété de cellules de son hôte après contact, non seulement avec la majorité des virus, mais encore avec des cellules et des endotoxines, des rickettsies, des nucléotides et un polysaccharide poly-anionique (Statolon). La production et l'activité de l'interféron exigent une nouvelle synthèse des protéines et de l'acide ribonucléaire cellulaires, bien que l'interféron produit *in vivo* par des endotoxines semble dépendre pour sa libération de l'existence d'un état préformé antérieur.**

**La pathogénie des infections virales primaires peut être largement fonction de mécanismes de défense indépendants de l'immunité. L'interféron, décelable dans le sérum de l'hôte et accompagnant les leucocytes durant l'évolution de la maladie virale, peut représenter une contribution importante à la guérison. Peu toxique, peu antigénique, doté d'une large activité antivirale, l'interféron est une possibilité thérapeutique intéressante. La stimulation de mécanismes autonomes générateurs d'interféron par des agents relativement inoffensifs peut avoir une action bénéfique chez l'homme.**

AT A special symposium on interferon sponsored by the American Society for Microbiology in May 1964[1] it was reported that certain bacteria and rickettsiae or products of nucleic acid hydrolysis could induce host cells to form interferon. This prompted the chairman of the symposium to enquire whether anyone knew of anything that did *not* stimulate production of interferon-like inhibitors.[1] His comment reflected the vast changes which have taken place in our understanding of this antiviral substance since it was first described in 1957 by Isaacs and Lindenmann[2] during studies on viral interference.

The term viral interference has been defined by Schlesinger[3] as "the inhibition of multiplication of one virus, or the suppression of injury, disease or death due to one virus in a host system simultaneously infected with another virus". Isaacs and Lindenmann[2] observed that fragments of chick chorioallantoic membrane incubated in the presence of heat-inactivated influenza virus A released a factor which could then be absorbed by fresh cells, rendering the latter relatively resistant to subsequent challenge with live influenza virus. This interfering agent was termed "interferon" and was provisionally considered to be an abortive product of virus multiplication. When the chorioallantoic cells liberating interferon were challenged with live virus, ensuing yields of new virus particles were appreciably below those from control cells, i.e. establishment of interference was accompanied by release of interferon.[2] Here, then, was a plausible explanation for other instances of viral interference, a subject extensively reviewed in recent years.[3, 4]

Various myxoviruses, arboviruses and poxviruses, either fully infectious or inactivated by physical means, were subsequently reported to produce in-

From the Virology Division, The Research Institute of The Hospital for Sick Children, Toronto, Ontario.
*Fellow of the Medical Research Council, Canada.

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terferons in eggs, laboratory animals, and tissue cultures of both primary and continuous lines. Much of this earlier work has been tabulated chronologically in a comprehensive article by Ho.[5] It is the purpose of this paper to review more recent studies on interferon which have led to current concepts regarding its physical and chemical nature, site and mode of action and its possible contribution to recovery from viral infections.

## PHYSICAL AND CHEMICAL PROPERTIES

Highly purified preparations of interferon from chick and mouse tissues have permitted accurate assessment of the nature and behaviour of this material.[6-9] It is a protein containing trace amounts of carbohydrate, with an isoelectric point at pH 6.75. Its activity is destroyed by proteolytic enzymes such as trypsin, chymotrypsin, pepsin and papain, but is not affected by peptidases, lipase, deoxyribonuclease, ribonuclease or neuraminidase. Structurally, chick interferon contains one or more polypeptide chains connected by and/or containing disulfide bridges essential for biologic activity.[9] The molecular weight of chick, mouse and human interferon determined by several different techniques is in the range of 20,000 to 38,000.[6-10] It is non-dialyzable through Visking tubing. The remarkable stability of interferon is evident from the fact that it withstands a pH range from 2 to 10 and can be stored for prolonged periods at 2°, —10°, or —70° C. Activity of chick interferon is retained after heating at 70° C. for one hour, whereas mouse and human interferon are somewhat more heat-labile.[11] Despite many similarities noted between purified mouse and chick interferon, a structurally dependent difference has been shown by precise gel filtration chromatography[8] and specific antibody studies.[12] Sensitivity to ether is variable.

It is important to emphasize that interferons produced in the same species of host cells *in vitro* or *in vivo* are identical following induction by viruses containing either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA).[7, 8]

## FACTORS AFFECTING PRODUCTION OF INTERFERON

### *Host Cells*

Interferon has been produced experimentally in cells of virtually every vertebrate examined, ranging from human leukocytes[13, 14] to kidney cells of the cold-blooded tortoise.[15] Chick interferon may be conveniently prepared by inoculation of influenza virus into the allantoic cavity of embryonated eggs previously incubated at 37° C. for nine to 13 days;[6] alternatively an arbovirus such as Chikungunya virus may be inoculated into chick embryo fibroblasts maintained in tissue culture.[16] Crude interferon is present in allantoic or tissue culture fluids collected after a further 24 to 48 hours' incubation. Serum and brain extracts of virus-infected mice contain large amounts of interferon.[17, 18] Continuous or stable lines of cells, including those originating from malignant tissue (e.g. HeLa), are also able to produce interferon in response to virus challenge.[19, 20]

Interferon appears to be a function of host cell maturation. Isaacs and Baron[21] found that chorioallantoic cells of 6-day-old chick embryos produced only about one-tenth as much interferon as cells of embryos five days older. Heineberg, Gold and Robbins[22] showed that with increasing age of mice the ability to form interferon increased, resulting in decreased multiplication of Coxsackie B1 virus and a corresponding reduction in mortality. Larke[23] inoculated chickens intravenously with massive doses of Powassan virus and found that peak titres of interferon in the serum of 6-day-old chicks were fourfold lower and occurred two to three hours later than maximum levels reached in 3-month-old birds. Intranasal infection with Sendai virus resulted in production of more virus but less interferon in 1-day-old mice compared with mice aged 4 weeks which were infected similarly.[24] However, Vilcek[25] reported that both virus and interferon titres were substantially higher in the brains of suckling mice dying two days after intracerebral infection with Sindbis virus than in adult mice, which rarely showed signs of illness.

### *Viruses*

Interferon production has been stimulated by a variety of RNA- and DNA-containing viruses, including those with oncogenic properties such as polyoma and Rous sarcoma virus.[5, 26-28] Adenoviruses may perhaps be excluded from the list.[29] The virus may be fully infectious or may be rendered inactive by exposure to ultraviolet light, heat or formalin. Influenza virus induces substantial yields of interferon under all of these conditions. Heat inactivation may enhance interferon production by Chikungunya virus but abolish the capacity to induce interferon formation by other group A arboviruses such as Mayaro and western equine encephalomyelitis virus.[30-32] Ho and Breinig[33] showed that inactivated Sindbis virus, added to cell cultures before inoculation of infectious virus, exerted a "priming" effect, i.e. interferon was produced under conditions where none was formed in the absence of the primer. Priming by inactive virus may also potentiate or increase interferon production where infectious virus alone is effective as an inducer.[34, 35] The reverse of this phenomenon is seen when interferon production by cells exposed to inactive virus is inhibited by superinfection with fully active virus. Lindenmann[36] has termed this "inverse interference", a possible explanation being the disruption of normal cellular biosynthetic pathways caused by the infectious virus.[34]

There are limitations upon any attempt to compare the interferon-inducing ability of one virus relative to others. Since variations occur from one virus-host system to another, such a system must

be clearly defined in each case. Gifford, Mussett and Heller[37] have shown that Chikungunya virus induced 70 times more interferon than did vaccinia virus or Newcastle disease virus under identical conditions in chick embryo fibroblasts. Ho[34] has drawn attention to the complex relationship which exists between Newcastle disease virus and various host cells: insignificant amounts of interferon are induced by this virus in tissue cultures of chick cells, whereas large amounts are produced in mouse cells. Appreciable amounts of interferon are not found in embryonated chicken eggs until 48 hours after infection with Newcastle disease virus, at which time the embryos are dead.[34]

Of practical significance may be the fact that avirulent vaccine strains of measles and foot-and-mouth disease virus induce more interferon than virulent, naturally occurring strains.[38, 39]

### *Other Variables*

The ratio of virus particles to host cells and temperature of the reaction have been found to influence interferon yields. Production is generally greater at temperatures above those optimal for viral growth, suggesting that fever accompanying viral infections may be of benefit in this regard.[40] It has been shown that a number of viruses with high optimal temperatures are the most virulent for the chick embryo, are poor producers of interferon and most resistant to its antiviral action.[41-43]

## RATE OF INTERFERON PRODUCTION

Heat-inactivated influenza virus produced interferon in chick chorioallantoic fragments three to six hours after inoculation.[2] Interferon was not detectable in cultures of chick embryo fibroblasts or L cells (a continuous line of mouse fibroblasts) infected with eastern equine encephalomyelitis virus, until 12 and 24 hours after inoculation, respectively. These times coincided approximately with the terminal phase of logarithmic virus replication.[44] Mice inoculated intravenously with very high doses of Newcastle disease virus showed interferon in serum one hour later, with peak titres by four hours—before any multiplication of injected virus took place.[17] These observations further illustrate the biological variation occurring within several different virus-host systems.

## INDUCTION OF INTERFERON BY NON-VIRAL AGENTS

Substances other than viruses have recently been shown to stimulate production of interferon-like material in certain host cells. Rotem, Cox and Isaacs[45] postulated that the entry of viral nucleic acid into a cell might trigger an interferon response since the nucleic acid was essentially "foreign" to that host. They showed striking inhibition of vaccinia virus when RNA from mouse livers (i.e. a "foreign" nucleic acid) was present in the nutrient medium of infected chick cell cultures.[45] Other workers subsequently demonstrated interferon in tissue cultures exposed to commercial-grade nucleic acids from thymus and yeast or even various nucleotides.[46, 47] Primary tissue cultures of chick embryo cells produced interferon when infected with *Rickettsia tsutsugamushi*.[48] Interest is currently focused on reports of interferon appearing in the serum of chickens, mice and rabbits as early as one or two hours after intravenous inoculation of large doses of bacteria or bacterial endotoxins.[49-51]

Statolon is an antiviral agent produced by the mold *Penicillium stoloniferum*. Structurally it is a complex anionic polysaccharide, thus sharing with RNA the properties of a polyanion. This similarity led to the demonstration by Kleinschmidt, Cline and Murphy[52, 53] that statolon stimulated interferon production in cultures of chick and mouse cells as well as *in vivo* following parenteral administration to mice.

Phytohemagglutinin is an extract of the kidney bean used in preparing cultures of leukocytes. As well as agglutinating erythrocytes present in the sample of peripheral blood, phytohemagglutinin has been shown to stimulate RNA and DNA synthesis in human leukocytes, increasing the mitotic rate of these cells. Wheelock[54] found that leukocytes from normal persons produced a virus inhibitor with interferon-like properties when cell cultures were incubated in the presence of phytohemagglutinin. The inhibitor appeared in the culture medium two hours after addition of phytohemagglutinin but could not be detected in the cell fraction itself.

It should be noted here that certain differences exist between "classical" interferons induced by viruses and those induced by endotoxins and statolon *in vivo*. Their significance will be discussed elsewhere in this review.

## ASSAY OF INTERFERON

Precise biochemical quantitation of interferon has not yet been achieved. Current methods of titration are based on the fact that interferon prevents or retards replication of challenge virus added to living cells. For reasons given above, the biological assay must be strictly defined in terms of host, period of preincubation with interferon, type and amount of challenge virus and temperature of reaction.

### *Reduction of Viral Hemagglutinin*

Fragments of chorioallantoic membrane from 10- or 11-day-old fertile hens' eggs are incubated in medium containing dilutions of material to be assayed for interferon. Membranes are subsequently challenged with influenza virus which produces hemagglutinin in proportion to the amount of viral replication taking place in the system. Cells protected by interferon do not support virus growth. Interferon can then be expressed in terms of the dilution of test material reducing the yield of

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hemagglutinin to an arbitrarily chosen fraction of that produced by controls incubated without interferon.[2, 55]

The same principle has been applied to the titration of calf interferon, using the Sendai strain of parainfluenza I as challenge virus in tissue cultures of calf kidney cells.[56]

*Quantitative Hemadsorption*

Tissue cultures infected with certain myxoviruses (e.g. influenza, parainfluenza) or arboviruses adsorb several species of erythrocytes to the surface of the cell monolayer; adherent red blood cells are readily observed under the microscope. This "hemadsorption" phenomenon can be measured quantitatively by releasing the hemoglobin from the erythrocytes and determining its concentration with a spectrophotometer. Finter[57] has developed this procedure into a sensitive and reproducible assay for interferon. Tissue cultures of suitable cells are incubated with the interferon preparation before challenge with a virus able to promote hemadsorption. Erythrocytes are added, then lysed with distilled water, permitting measurement of free hemoglobin. Cells protected by interferon support only limited growth of challenge virus, show reduced hemadsorption, and consequently yield less hemoglobin than control cultures.

*Virus Plaque Reduction*

Tissue culture monolayers are incubated with the interferon preparation, then challenged with a standardized dose of a plaque-forming virus. Following virus adsorption the cultures are overlaid with nutrient agar. The titre of interferon is expressed as the reciprocal of the highest dilution of interferon which reduces by 50% the number and size of virus plaques which develop in control cultures.[58]

Marked variation in sensitivity to interferon is observed among different viruses. Burke and Buchan[59] found Chikungunya and Bunyamwera viruses about four times more sensitive than Semliki Forest virus for titrating a single standard interferon preparation in chick embryo fibroblasts. The species of host cells used in the assay system also influences the choice of challenge virus. Eastern equine encephalomyelitis virus and vesicular stomatitis virus have been used extensively because of their ability to form distinct plaques, their sensitivity to interferon and the wide host range. Vaccinia virus forms plaques in chick embryo fibroblasts without the necessity of an agar overlay. This system has been studied in detail and applied to interferon assay.[60]

Interferon is rapidly absorbed by cells. Increasing the incubation period of cells and interferon from two or three hours to 24 hours before virus challenge enhances interferon titres only two- to four-fold.

*Indicator Test*

Cells incubated in small tubes (protected from the atmosphere with a layer of mineral oil) liberate acids in the course of normal metabolic activity; phenol red indicator in the culture medium turns yellow as the pH falls. Cells infected with virus are destroyed; the medium becomes alkaline and the indicator turns purple. Cultures preincubated with interferon survive virus challenge, the medium turning yellow as with normal cells. This method uses relatively few cells, permitting detection of smaller amounts of interferon. Using vesicular stomatitis virus as challenge, Paucker[12] has found this system highly reproducible and more sensitive than the plaque inhibition test.

*Reduction of Cytopathic Effects*

The replication of virus within susceptible cells frequently leads to degenerative changes. These "cytopathic effects" can be followed by microscopic observation of infected tissue cultures. Destruction is inhibited when cells are protected by interferon, which may be added to the system several hours before or at the same time as challenge virus. The versatility of this assay is evident from its application in tissue cultures of human, monkey, calf, chick and mouse cells.[6, 13, 54, 61, 62]

*Direct Comparison of Virus Yield*

Ribonucleic acid extracted from certain viruses can initiate the synthesis of new, complete virus particles when inoculated into host cells which do not otherwise support growth of that virus. Only one cycle of virus multiplication occurs under these circumstances. Hermodsson and Philipson[56] have assayed calf interferon by comparing the yields of poliovirus from calf kidney cell cultures, untreated or treated with interferon, followed by challenge with infectious poliovirus RNA. They report this method to be about 100 times more sensitive than assays based on percentage reduction of virus plaques or viral hemagglutinin.

## SPECIFICITY OF INTERFERON

Interferons produced in cells of a given host species have similar properties, even though inducing viruses may differ widely in chemical and biological characteristics. If the interferon is then added to other cells of that species, it will inhibit replication of a variety of challenge viruses, including the type which originally stimulated its production. The same interferon added to cells of a *different* host shows little or no antiviral properties. Host species specificity and lack of virus specificity are useful criteria differentiating interferon from other viral inhibitors. Purified or highly active crude preparations of mouse and chicken interferon showed no protective effects in heterologous cells, although absorption rates were similar in homologous and heterologous systems.[8, 63] Minor

degrees of cross-species activity reported by other workers,[62, 64] accompanied in some cases by different rates of absorption, may be due to non-specific cellular factors or residual interfering virus.[8, 63] Paucker,[12] in a series of elegant experiments, has shown that low-level protection of chick embryo cells by interferon produced in mouse fibroblasts was not neutralized by specific antiserum prepared against mouse interferon; the observed effects were therefore due to the presence of other components against which no antibodies were formed.

Exceptions to this specificity have been reported in genetically related species. Mouse interferon protected hamster and rat cells to a moderate degree, and human tissues have been protected by interferon produced in monkey cell cultures.[65]

## CHARACTERIZATION OF INTERFERON

A number of criteria, apart from species specificity, generally serve to distinguish interferon preparations from other inhibitors of viral activity:[6, 11, 17]

Interferon is (1) stable at pH 2; (2) moderately heat-stable (varies with species); (3) non-dialyzable; (4) not sedimented by centrifugation at 105,000 G for two hours; (5) inactivated by trypsin or other proteolytic enzymes; (6) unaffected by antibody to virus; and (7) free of residual viral particles capable of causing viral interference. (8) There is no direct inactivation of virus when the two are incubated together in a cell-free system. (9) Interferon cannot be washed off cells following absorption. (10) It is active against more than one type of virus.

Several "interferon-like" substances have been reported which differ only slightly from classical interferon. Inhibitors recovered from the cerebrospinal fluid of patients with viral or bacterial meningitis, or acute leukemia, were markedly inactivated upon exposure to pH 2.0 to 2.2 for 24 hours.[66] Instability at 56° C. has been reported for the antiviral substance appearing in the serum of rabbits receiving intravenous inoculation of the endotoxin or killed cells of *E. coli*,[51] and in the serum of two patients with meningitis and bacteremia caused by *H. influenzae*.[67] Similar heat lability in addition to instability at pH 2 and 10 was noted for the virus inhibitor produced by human leukocytes exposed to phytohemagglutinin.[54]

The molecular weight of interferon produced by injection of mice with bacterial cells or endotoxin was found to be two or three times greater than that of interferon induced by injection of virus.[68] Circulating interferon induced by statolon had an equally high molecular weight; however, in mouse embryo tissue cultures statolon-induced interferon had a lower molecular weight similar to other mouse interferon induced both *in vivo* and *in vitro* by virus.[69] These findings suggest that high-molecular-weight interferon produced *in vivo* is an aggregation of smaller molecules occurring either by polymerization with itself, or by aggregation or covalent bonding with other materials such as albumin.[68-70]

## PRODUCTION AND MODE OF ACTION

Earlier studies have shown that interferon does not affect extracellular virus, adsorption of virus to host cells, intracellular uncoupling of viral nucleic acid from its protein coat, or release of newly formed virus.[5] Various drugs, acting upon different phases of cellular metabolism, have helped to clarify mechanisms relating to the synthesis of interferon and the virus-inhibiting properties which it exhibits.

### *Inhibitors of DNA*

1. *Aminopterin.*—This folic acid antagonist inhibits new DNA formation without affecting RNA and protein synthesis.[71] It has no effect on interferon production in chick embryo tissue cultures.[72]

2. *Mitomycin C.*—This antibiotic of microbial origin creates a number of cross-linkages between the complementary helices of DNA, interfering with strand separation and therefore with new DNA formation. Mitomycin appears, however, to have little effect on its ability to direct the synthesis of messenger RNA for production of new protein, although these processes are eventually depressed because of breakdown of cellular DNA.[73, 74] Interferon synthesis is inhibited by mitomycin C, indicating that the process is dependent on intact host cell DNA.[72]

3. *5-Fluoro-2'-deoxyuridine (FUDR) and 5-Iodo-2'-deoxyuridine* (IDU).—These compounds are structural analogues of metabolic precusors in DNA synthesis. The latter is actually incorporated into DNA, forming a "fraudulent" molecule.[75] Levy, Axelrod and Baron[76] reported that FUDR did not affect induction of interferon by a DNA or RNA virus. However, Holmes, Gilson and Deinhardt[77] found reduced yields of interferon in mumps virus-infected tissue cultures treated with IDU. This conflicting evidence may be due to the higher levels of IDU employed, since with increasing concentration it can interfere directly with RNA synthesis.[72]

In summary, inhibition of DNA alone (by aminopterin or low doses of IDU) does not affect interferon production, while inhibitors of DNA which secondarily affect RNA synthesis (mitomycin C, high doses of IDU) also inhibit interferon. Therefore, new DNA formation *per se* is not required for interferon production but RNA synthesis is essential.[72]

### *Inhibitors of RNA*

1. *Actinomycin D.*—This drug specifically binds to the purine base, guanine, in DNA, impairing certain cellular functions of DNA without inter-

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fering with its synthesis. In low dosage actinomycin completely inhibits the production of all forms of cellular RNA (messenger, soluble or transfer, and ribosomal), since the RNA polymerase system is dependent upon fully functional DNA.[78, 79] Synthesis of viral RNA is not inhibited.[80] On the contrary, the growth of RNA viruses is enhanced in the presence of actinomycin D, probably because production of endogenous interferon is impaired owing to lack of its specific messenger RNA.[81, 82] The drug is no longer effective in blocking interferon production if added after more than about two hours of virus-cell interaction. These data strongly suggest that (i) interferon is a newly synthesized protein under the genetic control of cellular DNA rather than viral nucleic acid, and (ii) all the messenger RNA required for full yields of interferon is produced within about two hours of addition of virus.[83-85] Thus, interferon can be produced before significant amounts of new structural virus nucleic acid or protein are formed.[17]

*Inhibitors of Protein Synthesis*

1. *Puromycin.*—This agent interferes with protein synthesis by causing the enlarging polypeptide chain to disengage from the polysomal template before the protein molecule has been completed.[86]

2. *p-Fluorophenylalanine (FPA).*—This compound replaces phenylalanine during incorporation of amino acids into protein, resulting in formation of non-functional molecules.[87] Both the production and antiviral activity of interferon are inhibited by these two agents, indicating the requirement for new protein synthesis.[85, 88-91]

Based on the foregoing data, a working hypothesis for the synthesis of interferon and the mechanism of its antiviral effect can now be formulated:

1. Information for the interferon molecule is encoded within the genetic material (DNA) of normal, uninfected cells. This information is probably held "in check" by repressors.

2. Viruses (or other non-specific interferon inducers such as polyanionic molecules) entering the cell inactivate these repressors, permitting cellular DNA to form new messenger RNA bearing coded information for interferon synthesis.[52, 83, 92] Sufficient RNA for full yields of interferon is produced rapidly, probably within two hours.[84, 85]

3. The requirement for new protein continues as long as interferon is being produced, suggesting that an enzyme essential for interferon synthesis is formed.[90] Another possibility is that interferon is largely preformed, being activated by an intermediary compound which requires messenger RNA and new protein synthesis.[85]

4. Once interferon has been formed it may, in turn, act as the inducer of a specific cellular RNA, leading to the formation of a new antiviral protein. It has been proposed that this second protein may be an unstable enzyme which prevents the accumulation of new RNA, viral as well as host cell in origin. Since DNA viruses require RNA during replication,[93] this proposed mechanism could account for the inhibition by interferon of both DNA and RNA viruses. If this induced "second protein" were relatively labile, it would provide a self-limiting process allowing for recovery of normal RNA synthesis; it might also explain the time limitations of interferon activity.[88, 89, 91, 94-96]

EVIDENCE FOR PREFORMED INTERFERONS

Stinebring and Youngner[50] observed that injection of mice with Newcastle disease virus or *B. abortus* resulted in maximum virus-inhibiting activity in plasma 12 to 14 hours later. Following injection of certain other bacterial cells or endotoxin, the inhibitor was maximal at two hours, then rapidly declined. They speculated that the early appearing interferon was preformed in host cells, while late-appearing interferon was newly synthesized. By inhibiting protein synthesis in mice (using puromycin or cyclohexamide), they markedly suppressed levels of interferon resulting from injection of virus or *B. abortus* but were unable to block circulating interferon which followed injection of endotoxin.[97] The appearance of interferon in the absence of protein synthesis was interpreted as evidence that the inhibitor was in a preformed state and released from cells in response to the endotoxin. Marked differences in the molecular weight of interferon induced by these two mechanisms have been noted above.

Further support for the concept of preformed interferon comes from experiments by Ho and coworkers. Rabbits pretreated with actinomycin D or puromycin produced expected amounts of circulating interferon-like material in response to injection of bacterial endotoxin; interferon titres were greatly reduced following injection of virus. It was concluded that endotoxin released an interferon-like inhibitor which was independent of the transcriptive function of cellular DNA and new protein synthesis.[98, 99]

OTHER INHIBITORS OF INTERFERON

Hydrocortisone has been shown to decrease the production of interferon in eggs, tissue culture and mice.[100-103] The inhibitory effect was apparent only when hydrocortisone was preincubated with cultures for a period of 24 hours before virus challenge. Significant depression of interferon occurred whether infectious or inactivated RNA or DNA viruses served as inducing agents.[103] In contrast to actinomycin D, suppression of interferon yields in tissue culture was not accompanied by enhanced virus multiplication.[102, 103] Reinicke[101, 102] showed that chick embryo cells pretreated with interferon did not differ from normal cells in regard to adsorption and penetration of influenza

virus. Lower yields of interferon could not be attributed to impaired release secondary to drug-induced changes in cell membrane permeability.

It has been proposed that hydrocortisone might depress interferon synthesis because of its observed protein catabolic or anti-anabolic activity *in vivo*. However, other anabolic, androgenic and estrogenic steroids, as well as hormones primarily affecting electrolyte balance, inhibited interferon production in chick embryo tissue cultures.[104] Growth hormones which also increase protein synthesis *in vivo* had no influence upon yields of interferon. It appears that hormones of a certain molecular configuration have a marked, selective influence on protein synthesis in virus-infected cells, inhibiting production of interferon but not of viral proteins.[104]

Investigators disagree concerning the effect of steroid hormones upon the virus-inhibiting activity of interferon. Various reports present evidence for enhanced effect,[105] suppression,[100, 101] or no influence;[103, 106] discrepancies may perhaps be attributed to differences in experimental techniques.[106]

### INTERFERON AND VIRAL INFECTIONS

The mechanisms of antibody formation and delayed hypersensitivity are undoubtedly important factors influencing the outcome of repeated encounters between host cells and a given virus. Observations on the time of appearance and diffusion of antibody to the portal of entry, as well as the recovery from viral infections which can take place in the absence of detectable local or circulating antibody, suggest that the pathogenesis of primary viral infections is determined largely by non-immune host factors including interferon, elevated temperature, local acidity and local hypoxia. These problems have been extensively studied and reviewed by Baron[107] and others.[11, 108-111]

Interferon appeared rapidly in the serum of several experimental hosts injected intravenously with large doses of certain viruses.[17, 23, 49, 51, 112] Circulating interferon was strongly associated with moderate or high levels of viremia and not with virus growth in other tissues.[112] Passively injected or actively induced circulating interferon was found to protect mice against: (i) viruses injected into target organs such as the brain, (ii) intraperitoneal injection of a virus which multiplied at secondary peripheral sites following viremic spread, and (iii) intradermal injection of vaccinia virus. Thus, interferon produced rapidly after onset of viremia may spread in the blood stream to all parts of the body, impairing or inhibiting implantation of virus.[113] Finter[114, 115] found that interferon protected mice infected with Semliki Forest virus, an arbovirus which produced high titres of viremia beginning as early as four hours after intraperitoneal inoculation. As a rule, interferon actively induced by intravenous injection of Newcastle disease virus was more effective than passive administration of interferon produced in mouse brains. Interferon given intravenously was more protective than intramuscular interferon even when injection by the former route took place four hours after challenge with Semliki Forest virus. Presumably the immediate high levels of interferon achieved were able to protect capillary endothelial cells of the brain and other tissues before the onset of viremia.[115, 116]

Interferon has been reported in the serum of humans during clinical viral infections and following exposure to standard live virus vaccines.[117-120] Peak interferon production occurred six days after vaccination of adults with the 17-D strain of yellow fever virus; viremia was maximal on the fifth day. The appearance of antibody within 24 hours after interferon prevented assessment of their relative roles in terminating the viremia.[118] Volunteers infected intranasally with influenza A2 virus developed interferon in nasal washings and serum between two and nine days after vaccination. Virus shedding preceded the appearance of interferon and frequently continued after interferon was no longer detectable.[119] Petralli, Merigan and Wilbur[120, 121] found interferon in the serum of children six to 11 days after primary measles vaccination with live attenuated virus; peak titres were observed on the ninth and tenth days and seemed to parallel the febrile response. Children subsequently inoculated with smallpox vaccine nine to 15 days after measles immunization failed to show primary "takes", indicating that circulating interferon induced by the live measles virus was associated with systemic protection against the totally unrelated vaccinia virus.[121]

A phase of generalized viremia occurs during the course of aseptic meningitis. The interferon-like substance recovered from cerebrospinal fluid of patients with this disease perhaps reached the central nervous system from the blood.[66]

### RELATIONSHIP BETWEEN LEUKOCYTES AND INTERFERON

Viruses have been isolated from the leukocyte fraction of peripheral blood; it is of interest, then, that *in vitro* preparations of human and animal leukocytes release interferon following viral infection.[13, 14, 122-125]

Glasgow and Habel[124] induced interferon in peritoneal leukocytes of mice by injecting inactivated vaccinia virus. Animals were significantly protected when infected intracerebrally with lethal doses of vesicular stomatitis virus 24 hours later. Presumably, interferon-producing leukocytes may be transported in the circulation to protect distant sites from viral infection.

Virus which is not rapidly adsorbed from the circulation following a sudden massive viremia has been associated with the leukocyte fraction of blood;[123] these leukocytes may produce the interferon found in the serum of hosts injected intravenously with massive doses of virus.[17, 23, 50]

Further evidence of the protective effects of leukocyte-induced interferon has recently been presented using an *in vitro* model which was therefore not subject to immune mechanisms of defence.[126]

Other tissues rich in reticuloendothelial cells, such as liver and spleen, also produce significant titres of interferon following virus infection.[125, 126]

## INTERFERON AS A CHEMOTHERAPEUTIC AGENT

The wide spectrum of antiviral activity, low toxicity and weak antigenicity of interferon have led to hopes that this naturally occurring defence mechanism might be exploited for human use. Early reports were encouraging. Interferon produced in monkey kidney tissue cultures was injected into the skin of human volunteers and followed 24 hours later by smallpox vaccination at the same site. A primary "take" was prevented in 24 of 38 cases.[127] The epithelial stages of human vaccinial keratitis were found to regress following topical application of interferon.[128] A recent trial has studied the effect of interferon on a number of respiratory virus infections. Volunteers received interferon as nasal drops or spray 11 times a day. Lack of protection was attributed to the low potency of the preparation and its rapid elimination by the mucociliary blanket of the nasal epithelium.[129] Studies reported to date have employed monkey interferon, which may partially explain the disappointing results.

Present hope appears to lie in stimulating endogenous production of interferon by administration of relatively innocuous viruses or non-specific inducers such as the polysaccharide statolon.[29, 130] The detection of circulating interferon following repeated administration of viruses to a patient with acute leukemia indicates the feasibility of this approach.[131]

## SUMMARY

Interferon is a naturally occurring antiviral substance produced by host cells following exposure to a wide range of infectious or inactivated viruses. Purified preparations indicate that it is a relatively stable protein of low molecular weight. Interferon inhibits, to varying degrees, most animal viruses, but this property is essentially restricted to cells of the species from which the interferon was derived. Certain bacteria, endotoxins and polyanionic substances have recently been shown to induce interferon-like material *in vivo* and *in vitro*.

The production and mode of action of interferon have been studied using drugs which selectively inhibit synthesis of cellular DNA, RNA or protein. A currently accepted hypothesis of interferon mechanisms is presented. Interferon induction by non-viral agents *in vivo* involves different metabolic pathways, suggesting that the interferon is present in a preformed state.

The pathogenesis of primary viral infections may be determined largely by the host's non-immune defence mechanisms, including the rapid formation of circulating interferon. The particular role of leukocytes and other tissues rich in reticuloendothelial cells is discussed. Activation of the host's inherent interferon-producing system by administration of relatively innocuous inducing agents may be a feasible approach to the treatment of human viral infections.

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